

## RESEARCH ARTICLE

# Impact of Foliar Bio-Based Fertilization on Spring Wheat (*Triticum Aestivum* L.) in Reconciling Productivity and Alleviation of Environmental Concerns Under Semi-Arid Conditions

Gani Stybayev<sup>1</sup>, Raushan Ramazanova<sup>2</sup>, Kelvin Harrison Diri<sup>1\*</sup>, Aliya Baitelenova<sup>1</sup>, Meisam Zargar<sup>3</sup>, Nogayev Adilbek<sup>1</sup>, Kulzhabayev Yeldos<sup>1</sup>

<sup>1</sup>Department of Plant Production, Faculty of Agronomy, S. Seifullin Kazakh Agrotechnical Research University, Astana 010000, Kazakhstan

<sup>2</sup>U.U. Uspanov Kazakh Research Institute of Soil Science and Agrochemistry, Almaty 050060, Kazakhstan

<sup>3</sup>Department of Agrobiotechnology, Institute of Agriculture, RUDN University, Moscow 117198, Russia

\*Corresponding Author: [kelvin.h.diri@kazatu.edu.kz](mailto:kelvin.h.diri@kazatu.edu.kz)

**Abstract:** A two-year randomized field trial was conducted to evaluate the effects of foliar bio-based fertilization on the growth, productivity, grain quality, and soil health of spring wheat (*Triticum aestivum* L.) under semi-arid conditions in Northern Kazakhstan. Four treatments were compared: T0 (100 kg Ammophos, mineral fertilizer control), T1 (50 kg N<sub>14</sub>P<sub>23</sub> + biotreatment), T2 (25 kg N<sub>14</sub>P<sub>23</sub> + biotreatment), and T3 (biotreatment only), where biotreatment consisted of biosulfur organic fertilizer and amino-acid biostimulants applied foliarly at 1 L/ha. Growth parameters, including plant height, root length, plant density, and productive tiller count, were significantly improved across all biotreated plots relative to the control. T1 achieved the highest biological and grain yields at 28.2 and 24.3 dt/ha, respectively. Grain protein content was significantly elevated in all experimental treatments. Normalized Difference Vegetation Index (NDVI) values were consistently higher in T1 and T2 at all measured growth stages, indicating improved chlorophyll content and overall plant health. Soil microbial activity, assessed via fluorescein diacetate (FDA) hydrolysis, was highest in T2, reflecting enhanced enzymatic function and soil biological activity under reduced mineral fertilizer input. Overall, T1 (50 kg N<sub>14</sub>P<sub>23</sub> + biotreatment) is recommended as the most effective strategy, combining high productivity, improved grain quality, and enhanced soil health while reducing dependence on conventional mineral fertilizers. These findings support the potential of bio-based foliar fertilization as a sustainable nutrient management approach for spring wheat production in semi-arid environments.

**Keywords:** Spring wheat, *Triticum aestivum*, Biostimulants, Bio-based fertilization, Soil microbial activity, NDVI, Grain quality, Semi-arid conditions

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## Introduction

Sustainable agriculture is an essential approach to food production that seeks to balance environmental health, economic profitability, and social responsibility. However, with the growing global demand for food and the increasing challenges posed by climate change and soil degradation, the need for sustainable farming practices has become more urgent than ever [1]. Kazakhstan ranks among the world's leading wheat producers, with more than 14 million tons of wheat produced annually, the majority of which is spring wheat [2] cultivated on approximately 13.5 million hectares of agricultural land. This intensive agricultural system, however, is characterized by the widespread use of mineral fertilizers, which has become a notable trend. Although several research studies [3] have highlighted the efficiency of fertilizers in improving growth and yield particularly in the northern regions, grain yields in Kazakhstan remain low; for instance, the average grain yield ranges between 10-13 c/ha compared with global average of 3.3t/ha (33c/ha) [4, 5].

This decline in yields is attributed to unfavorable climatic conditions, particularly uneven precipitation and limited water availability, even during summer [6]. These factors contribute to restricted nutrient uptake and reduced Fertilizer Use Efficiency (FUE), leading to yield variations [7]. In addition to yield declines caused by these edaphic factors, the continuous use of mineral fertilizers raises soil fertility concerns, including ecological damage [8], soil acidification, reduced humus content, and a decline in both total and mobile nitrogen, as reported by Saparov [9]. This underscores the importance of adopting a new approach centered on sustainable and environmentally friendly agricultural practices. In this context, the application of biostimulants and bio-organic fertilizers is favored as a strategy to enhance crop productivity while preserving and improving soil fertility.

Their use as an alternative to chemical fertilizers plays a crucial role in promoting soil sustainability. They enhance soil fertility and crop yields while being environmentally friendly, serving as a renewable source of plant nutrients [10], and minimize the reliance on synthetic fertilizers. This is evident in a study by Lamlom et al., who observed an increase in both grain and biological yield of spring wheat incorporating biofertilizers with mineral fertilizers [11]. This significantly increased grain and biological yield while growth parameters including 1000-seed weight, harvest index (H.I), increased and differed significantly.

Biostimulants play a crucial role in promoting plant growth and enhancing stress tolerance, particularly in arid and semi-arid environments where crops are frequently exposed to abiotic stressors [12]. These inputs are broadly categorized based on their composition, including amino acid-based formulations, seaweed extracts, and humic or fulvic acids. In addition to their direct impact on plant physiology, biostimulants contribute to improved soil biological health by stimulating microbial activity and enhancing biochemical processes within the rhizosphere [13].

Among these, amino acid-based biostimulants, typically derived from plant or animal proteins through enzymatic or chemical hydrolysis have received considerable attention due to their multifaceted agronomic benefits. A growing body of research supports their use in modern crop management. For instance, foliar application of amino acid-based products have been shown to increase yield and grain quality in rice [14], while also enhancing physiological traits and productivity in winter wheat under field conditions [15, 16].

Despite the demonstrated potential of biostimulants and bio-organic fertilizers in improving crop performance and supporting environmentally sustainable farming systems, their widespread adoption in Kazakhstan remains limited. This mainly reflects the dominance of conventional chemical fertilizers in local agronomic practices. In light of the pressing need to reduce dependence on mineral fertilization, this study investigates an integrated approach of low dose mineral fertilization, foliar applied organic sulfur fertilizer and amino-acid based biostimulant with the aim of improving the growth, yield, and grain quality of spring wheat. For this study, we hypothesized that reducing the fertilization dose combined with the application of these biotreatments could enhance wheat growth and yield while maintaining soil health and fertility.

## Materials and Methods

### Site and Climate Description

The experiment was carried out at the Zaporozhye Agro experimental field located in Zhaksynsky district, Akmola region with coordinates 51°16'12" N, 70°28'30" E, 375m above sea level. The study area exhibits a sharply continental and arid climate, characterized by substantial annual and diurnal temperature fluctuations. During the 2023 growing season (April to October), maximum air temperatures ranged from 17°C in April to a peak of 40°C in July, before gradually declining to 25°C

in October. Minimum temperatures followed a similar trend, increasing from approximately 0°C in April to 30°C in August. Monthly precipitation levels were irregular, ranging from 9 mm in May to 74 mm in September. Notably, the highest precipitation was recorded in August (70 mm), followed by September and July with 74 mm and 27 mm, respectively. Lower rainfall occurred in April (17 mm), May (9 mm), June (12 mm), and October (31 mm).

In the 2024 growing season, temperature patterns showed a similar profile. Maximum temperatures rose from 25°C in April to 36°C in July, before decreasing to 24°C in October. Minimum temperatures increased from 5°C in April to a peak of 26°C in August. Precipitation varied significantly, ranging from just 8.4 mm in September to a maximum of 100 mm in August. Other notable values include 63 mm in April, 36 mm in June, and 40 mm in July. Lower precipitation was observed in May (25 mm) and October (12 mm), while total mean precipitation for both years (2023, 2024) were 240 and 284mm respectively.

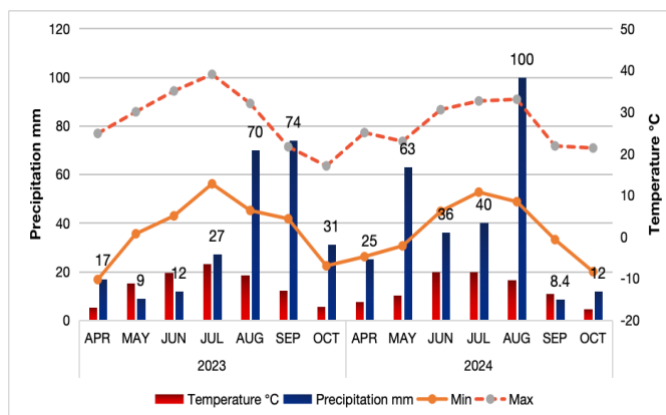


Fig. 1: Agrometeorological conditions during the study at Zaporozhye experimental field 2023-2024

## Experimental Design and Treatments

The field experiment was conducted on a 0.075 ha plot, following a single-factor Completely Randomized Design (CRD) to ensure unbiased allocation of treatments. Three treatment formulations were tested: T1 (50kg N<sub>14</sub>P<sub>23</sub> + Biotreatment), T2 (25kg N<sub>14</sub>P<sub>23</sub> + Biotreatment), T3 (0kg N<sub>14</sub>P<sub>23</sub> + Biotreatment) based on Koppert’s fertilization scheme. Each plot measured 5m × 10m and was replicated three times. All wheat seeds, except those in the control treatment, were treated with Seedspor-W, a microbial biostimulant containing Arbuscular mycorrhizal propagules and Bacillus spp. In addition, biotreatments T1, T2 and T3 were developed by combining Seedspor-W with recommended doses of Hansebiosulfur (a micronized liquid fertilizer containing 70% elemental sulfur derived via microbial synthesis) and HanseAmino (a plant-derived amino acid biostimulant produced through enzymatic hydrolysis), along with 50 kg and 25 kg doses of starter NP mineral fertilizer. All biostimulants and bio-derived fertilizers were applied at rates recommended by the manufacturer (HansePlant).

These treatments were designed to assess the combined effects of reduced mineral dose and biopreparations on soil health and crop performance. 100kg of Ammophos (MAP) fertilizer was used as control (T0) treatment as it represents the standard fertilizer program on the farm as a baseline to compare the effectiveness of the biofertilizer. Biotreatments were also applied foliarly and through soil drenching to maximize nutrient uptake and improve rhizosphere conditions.

Table 1: Description of control and biotreatment formulations used in the experiments during 2023 and 2024 cropping seasons

Treatments	Composition
T0 (control)	100kg Ammophos fertilizer
T1	Seedspor-W+Smart Start N <sub>14</sub> P <sub>23</sub> (50kg/ha <sup>-1</sup> ) + HanseAmino and Hansebiosulfur (1 L/ha)
T2	Seedspor-W+Smart Start N <sub>14</sub> P <sub>23</sub> (25kg/ha <sup>-1</sup> ) + HanseAmino and Hansebiosulfur (1 L/ha)
T3	Seedspor-W+Hanse Amino and Hanse biosulfur (1 L/ha)

**Table 2: Composition of Biotreatment products used in the experiments during 2023 and 2024 cropping seasons**

Seedspor-W	Hanse Amino	Hanse Biosulfur
<i>Trichoderma asperellum</i> 2.10 spores/ml	Total amino acid 24%	70% micronized sulfur
<i>Arbuscular mycorrhizal</i> propagules 10 colonies/ml	Free amino acid 13%	
Bacillus 2.10 spores/ml	dry weight 32%	
Organic matter 89.2%		
Organic carbon 70.0%		
P <sub>2</sub> O <sub>5</sub> 0.1%		
K <sub>2</sub> O 2.0%		
MgO 0.1%		
Calcium 0.15%		
Iron 2%		

### Land Preparation and Sowing Procedure

During autumn experiments, the primary soil cultivation was conducted using deep loosening to a depth of 22–25 cm with a STAVR PG 3 and 5 deep tillers to enhance soil aeration and structure. In spring 2024, field surface leveling was performed, and weed seedlings were controlled using heavy-toothed harrows (BZTS-1.0), cultivating the top 4–5 cm of soil.

The soil in the experimental field was classified as ordinary Chernozem, typical of soils in Northern Kazakhstan, having a silty clay texture with a pH 7.0, 4 % humus content, optimum nitrate nitrogen (15.4mg/kg), available phosphorus content (18.3mg/kg) and characterized by high exchangeable potassium concentration (311mg/kg) [17]. Details of the soil characteristics of the study area are given in Table 3.

**Table 3: Soil properties of the experimental field**

Soil properties	Unit	Value
pH		7.0
Humus	%	4
Nitrate nitrogen	mg/kg	15.4
Available phosphorus	mg/kg	18.3
Exchangeable potassium	mg/kg	311
Moisture content	%	58

### Soil and plant analysis

Soil samples were collected randomly from each plot at 20cm depth using a bucket auger. Samples were oven dried at room temperature and ground to < 2mm using a mechanical sieve. Soil pH was then assessed using a soil-to-water suspension at a 1:5 weight-to-volume (w/v) ratio. The concentrations of available phosphorus and exchangeable potassium in the soil were quantified using the Machigin method, as modified by TsINAO (GOST 26205-84). The humus content in the arable soil layer was determined using the Tyurin method in accordance with GOST 26213-91. This method provides an accurate assessment of soil organic matter by oxidizing organic carbon and measuring its content, ensuring reliable evaluation of soil fertility and quality. The nitrate nitrogen (N-NO<sub>3</sub>) content was determined through the ionometric method, which provides precise measurements of nitrate levels in the soil.

Soil enzymatic activity was assessed using the Fluorescein Diacetate (FDA) hydrolysis assay, following the standard procedure described by Adam and Duncan [18]. This method measures the overall microbial activity based on the hydrolytic cleavage of fluorescein diacetate by nonspecific esterase's, proteases, and lipases present in active microbial communities.

To evaluate wheat growth, key agronomic parameters were recorded, including plant height (cm), total plant biomass, number of grains per spike, spike length, and spike weight, all of which contribute to understanding crop development. Additionally, wheat yield and yield components were also computed, containing grain yield, biological yield (expressed in

centers per hectare (c/ha)), 1000-grain weight. Harvest index provides insights into the overall productivity and efficiency of the wheat crop under the experimental conditions and was calculated as:

$$\text{Harvest index (H.I)} = \text{Grain yield} \times 100 / \text{Biological yield} \quad (1)$$

## Protein and Gluten Content

Grain samples were taken from each experimental plot assigned different treatments, dried and crushed to determine total nitrogen (%). 0.5g crushed grain was collected and we-digested with a mixture of H<sub>2</sub>SO<sub>4</sub> - H<sub>2</sub>O solution, and Total-N was calculated calorimetrically using Nessler's approach. Measurements were taken at 420nm, and %N calculated using the formula;

$$\text{Percentage nitrogen} = \% \text{NH}_4 \times 0.78 \quad (2)$$

Protein content was further calculated using the formula:

$$\text{Total protein} = \% \text{ nitrogen content} \times 6.25 \quad (3)$$

Gluten values in grains were determined via glutomatic 2200 apparatus using NaCl solution [19].

## Data Analysis

All plant and soil data were subjected to two-way Analysis of Variance (ANOVA) using Minitab version 17. Significant differences among treatment means were determined using Tukey's post-hoc test at a 95% confidence level ( $p < 0.05$ ). Principal Component Analysis (PCA) was performed using R-studio to identify patterns and relationships among measured traits, reduce dimensionality, and visualize the clustering of treatments based on multivariate responses. PCA biplots were used to interpret the contribution of individual variables to treatment differentiation and to better understand the underlying interactions among agronomic and soil health parameters.

## Results and Discussion

### Spring Wheat Growth Parameters and NDVI Influenced by Biotreatments

A statistically significant improvement ( $p < 0.05$ ) was observed in plant height across all treated plots compared to the control T<sub>0</sub>, which recorded the lowest mean value (79 cm). The T<sub>1</sub> (50kg N<sub>14</sub>P<sub>23</sub> + Biotreatment) caused the highest plant heights (87.3 cm), followed by T<sub>2</sub> (25kg N<sub>14</sub>P<sub>23</sub> + Biotreatment) (84.7 cm) and T<sub>3</sub> (0kg N<sub>14</sub>P<sub>23</sub> + Biotreatment) (83.3 cm); however, the differences among treatments were not statistically significant (Table 4).

Productive tillering and stem number were most enhanced with the T<sub>1</sub> treatment, which combined a low dose of mineral NP fertilizer with the foliar-applied biosulfur and amino acid-based biostimulants. Both T<sub>1</sub> and T<sub>2</sub> positively affected plant density and root length, achieving the highest values (301 and 298 plants/m<sup>2</sup>, 21.2 and 20.3 cm, respectively), while the control exhibited the weakest performance. These improvements likely resulted from the synergistic effects of nutrient priming (via starter N<sub>14</sub>P<sub>23</sub>), biostimulant-induced root stimulation, and improved soil biological interactions. This finding aligns with the results of Alobaidy et al., who reported that applying 50% of the recommended fertilizer dose in combination with biofertilizer had the most pronounced effect on plant growth [20].

Treating wheat seeds with Seedspor-W, which contains Arbuscular Mycorrhizal Fungi (AMF) before sowing, significantly enhanced root development, through mutualistic associations with the plant roots, improving water and nutrient uptake, particularly phosphorus required for root development. Prior research confirms that such microbial inoculants stimulate root proliferation and improve soil structure [21-23].

**Table 4: Impact of bio treatments on morphological traits of spring wheat, average data for 2023 -2024**

Treatment	Plant height	Plant number	Productive stems	Productive tillers	Root length
T <sub>0</sub>	79±1.0b	283±0.5c	315±1.5c	310±0.0c	15.4±0.5b
T <sub>1</sub>	87.3±1.3a	301±0.6a	365±2.3a	337±0.0a	21.2±0.3a
T <sub>2</sub>	84.3±0.2a	298±1.4a	345±6.3b	323±0.0b	20.3±0.7a
T <sub>3</sub>	83.7±1.9a	289±2.6b	333±10.0b	317±0.0bc	17.2±0.9b

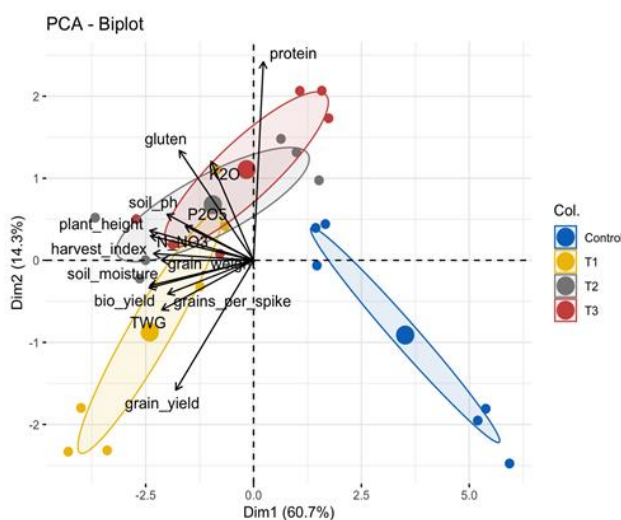
t-test	**	**	**	**	**
p<0.05	0.001	0.002	0.001	0.001	0.003

Mean±SD with different letters are significantly different, \*\*significant at p<0.05, ns: not significant

**Table 5: Biotreatment effect on yield components including Biological Yield (B.Y), Grain Yield (G.Y) harvest index (H.I), Thousand Seed Weight (TSW), Grains Per Spike (GPP) and Grain Weight (GW) of spring wheat, average data for 2023 -2024**

Treatment	BY	GY	HI	TSW	GPP	GW
T0	19.9±0.2c	15.3±1.1c	76±0.0b	35.6±0.5a	19±0.5b	0.78±0.0a
T1	28.2±1.1a	24.3±1.0a	86±0.0a	36.2±0.1a	22±1.3a	0.84±0.0a
T2	24.8±0.0b	21.2±0.1ab	85±0.0a	35.8±0.1a	21±0.0ab	0.78±0.0a
T3	24.6±0.9b	20.8±0.9b	84±0.0a	35.4±0.0a	20±0.3b	0.80±0.0a
t-test	**	**	**	ns	**	ns
p<0.05	0.001	0.001	0.001	0.075	0.008	0.128

different, \*\*significant at p<0.05, ns: not significant



**Fig. 2: PCA of Traits Under Biotreatments**

Additionally, the inclusion of amino acid-based biostimulants likely supported nitrogen assimilation, protein synthesis, and cell expansion, while biosulfur may have contributed to enhance photosynthetic efficiency and root metabolic activity due to sulfur's role in amino acid and enzyme synthesis [24]. These mechanisms align with findings by Desai et al. and Dal Cortivo et al. who observed improved root and shoot growth in wheat under integrated nutrient management strategies. Importantly, root length was not only improved by biostimulant application, but also by the presence of mineral phosphorus, known to promote root elongation during early development [25-27]. Thus, the observed increases in vegetative growth metrics can be attributed to the combined action of microbial symbiosis, targeted biostimulant activity, and essential starter fertilization.

Normalized Difference Vegetation Index (NDVI) is a pivotal index for assessing crop vigor, chlorophyll content, and biomass accumulation which offers deeper insight into proper plant management strategies. All bio treatments enhanced NDVI values across key growth stages compared to the control plot ( $p < 0.05$ ). At the seedling stage, the control recorded the lowest NDVI (0.13), reflecting poor early vigor. T1 (0.21) and T2 (0.20) treatments showed markedly higher values, likely due to early nitrogen metabolism and root stimulation supported by the applied biostimulants.

During tillering, NDVI values rose across all treatments, with T1 (50kg N<sub>14</sub>P<sub>23</sub> + Bio treatments) (0.43) and T2 (25kg N<sub>14</sub>P<sub>23</sub> + Bio treatments) (0.40) outperforming the control (0.31), consistent with increased vegetative growth and nutrient uptake. At heading, T1 and T2 reached NDVI values of 0.65 and 0.60, significantly higher than control (0.47). The sustained NDVI values as the plant progresses into heading stage suggests enhanced chlorophyll concentration, nutrient availability, and photosynthetic efficiency [28-30]. However, this trend contrasts with previous findings by Hnizil et al., who recorded decline in

NDVI values in wheat during heading stage following the application of mineral nitrogen fertilizers at different rates, attributing this to a transition from vegetative growth to reproductive development and grain filling, a response seemingly mitigated in the present study by the integrated use of biofertilizers and reduced mineral inputs [31].

A possible explanation lies in the mode of action of biostimulants and bio fertilizers compared to mineral nitrogen fertilizers. While mineral nitrogen fertilizers can initially boost vegetative growth, biostimulants and bio fertilizers rich in amino-acid and Sulphur promotes sustained chlorophyll retention and improved photosynthetic activity as observed in our results [32, 33].

## Yield and Yield Components of Spring Wheat

Data analysis revealed that foliar application of biotreatments had a significant effect on the yield and yield components of spring wheat. Both biological and grain yields followed the trend: T1 > T2 > T3 > T0 (Control). The T1 resulted as the highest biological and grain yields, recording 28.2 and 24.3 c/ha respectively, both significantly higher ( $p < 0.05$ ) than those observed in the control plot (19.9 and 15.3 c/ha). Although T2 and T3 also led to significant yield increases compared to the control ( $p < 0.05$ ). The difference however between them was not statistically significant ( $p > 0.05$ ) (Table 5).

These findings are consistent with Narula et al., who observed enhanced grain yield in spring wheat following the use of biofertilizers and seed inoculation [34]. Similar results were reported in studies by Karimi et al. and Hojattipor et al. where the integration of Arbuscular Mycorrhizal Fungi (AMF) seed treatments, low-dose mineral fertilization, and foliar biostimulants contributed to improved yield performance [35, 36]. Furthermore, the increased grain yield observed in the treated plots may be attributed not only to improved soil moisture retention compared to the control, but also to greater precipitation during the 2024 season. Together, these factors likely enhanced nutrient solubility, uptake efficiency, and overall crop productivity [37, 38].

In figure 2, Principal component analysis PC1 and PC2 explained over 70% of the total variance in the dataset. The biotreatments clustered into four distinct groups, highlighting their varied influences on crop traits. T1 (50kg N14P23+ Biotreatment), which achieved the highest grain and biological yields, was closely associated with core productivity indicators grain yield, biological yield, grains per spike, and 1000-grain weight demonstrating its strong contribution to overall crop performance. This supports the findings of Al-Suhaiban et al., where grain yield showed strong association with the combined application of 50% NPK and biofertilizers [39].

T2 (25kg N<sub>14</sub>P<sub>23</sub> + Biotreatment) clustered with plant height, harvest index, and nitrogen uptake traits (soil pH and N-NO<sub>3</sub>), indicating moderate effects on vegetative growth and nutrient dynamics, but lower yield impact than T1. T3 (Biotreatment only) was linked with grain quality traits (protein, gluten) and soil moisture, suggesting benefits for nutritional value and soil condition rather than yield. In contrast, the Control showed minimal association with any major traits, confirming its poor performance.

The harvest index, a key indicator of a plant's efficiency in allocating assimilated resources toward grain production, was significantly influenced by the biotreatments ( $p < 0.05$ ). The T1 biotreatment recorded the highest harvest index (86%), followed closely by T2 (85%) and T3 (84%), while the control (T0) showed the lowest value (76%) (Table 5). This improvement suggests more effective partitioning of biomass toward reproductive structures in treated plants. However, no statistically significant differences ( $p > 0.05$ ) were observed among the biotreatment groups (T1, T2, and T3), indicating comparable performance in promoting biomass partitioning. A similar outcome was reported by Ahmed et al., who also found no significant variation in harvest index among different biotreatment formulations despite overall improvement relative to untreated controls [40]. Although no statistically significant differences ( $p > 0.05$ ) were observed for 1000-grain weight and seed weight per spike across treatments. The number of grains per spike was obtained favorably higher for T1, compared to T2 and T3 (Table 5).

## Protein, Gluten and Moisture Content of Wheat Seeds

It is clear from the results in table 6 that bio treatments had a significant impact on wheat grain quality, most notably enhancing protein content. While grain moisture content and gluten content remained statistically unchanged across treatments, a significant increase in protein content was observed in all bio fertilized plots compared to the control ( $p < 0.05$ ). Specifically, the control group exhibited a protein level of 12.37 %, whereas all bio fertilized groups showed increased values ranged 13.8% to 14.2%.



**Table 6: Bio treatment effect on protein, gluten and moisture content of wheat seeds during 2023 and 2024 cropping seasons**

Treatment	Protein content %	Gluten content %	Moisture content %
T0	12.37±1.4b	29.5±0.4a	13.3±0.0a
T1	14.2±0.3a	30.7±0.3a	13.0±2.0a
T2	14.0±0.1a	30.2±0.4a	13.5±0.0a
T3	13.80±0.0a	29.9±0.3a	13.4±0.0a
t-test	**	ns	ns
p<0.05	0.001	0.116	0.572

Mean±SD with different letters are significantly different,\*\*significant at p<0.05, ns: not significant

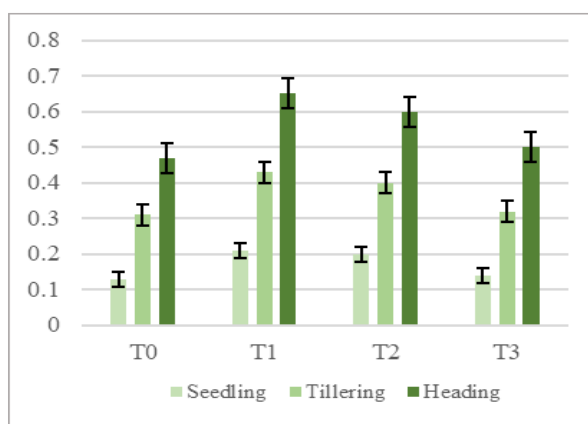
Protein accumulation in wheat seeds is likely attributable to the use of amino acid biostimulants as well as biosulfur, both of which contain appreciable amounts of sulfur and nitrogen, which are key elements in protein synthesis. This corroborates Popko et al., where protein content in wheat increased on application of elevated doses of amino-acid based biostimulants [41]. This suggests that there is practical potential for further improving grain protein content through optimized application rates of bio stimulant, warranting additional dose–response studies to explore this prospect under varying agronomic conditions. The lack of significant differences in gluten content, despite changes in protein levels, suggests that the composition or proportion of gluten-forming proteins (gliadins and glutenins) may not have been altered substantially by the bio treatments. It is however important to note that both T1 and T2 bio treatments, slightly increased gluten content. This was further corroborated by Cortivo et al., who observed no significant effect on gluten content and grain quality in bio fertilized fields [42].

**Table 7: Biotreatment effect on Soil properties**

Treatment	Soil pH	Soil moisture (%)	N-NO <sub>3</sub> (mg/kg)	P <sub>2</sub> O <sub>5</sub> (mg/kg)	K <sub>2</sub> O (mg/kg)
T0	6.15±0.0b	58±1.5c	21±1.0a	18.3±0.3c	311±1.0b
T1	7.41±0.0a	65±1.0a	18±0.2b	22.4±0.3a	324±12.2a
T2	7.44±0.0a	63±0.3b	13±0.4c	21.3±0.2ab	324±12.2a
T3	7.04±0.4a	62±1.6b	11±0.3d	19.6±0.7b	325±12.0a
t-test	**	**	**	**	ns
p<0.05	0.001	0.010	0.001	0.001	0.050

Mean±SD with different letters are significantly different,\*\*significant at p<0.05, ns: not significant

Moisture content remained within a narrow range (13.0 – 13.5%) and did not differ significantly among treatments. Maintaining uniform moisture content is crucial for post-harvest stability and processing, and these results indicate that bio fertilizer and biostimulants did not interfere with this parameter, a favorable outcome for grain storage and industrial use.



**Fig. 3: Biotreatment effect on NDVI variation across developmental stages of spring wheat during 2023 and 2024 cropping seasons**

## Biotreatment Effect on Soil Chemical Properties

Bio treatments significantly affected key soil chemical properties, indicating enhanced fertility and nutrient availability, both critical for sustainable crop production. Statistically significant differences ( $p < 0.05$ ) among treatments were observed in humus content, pH, moisture, nitrate nitrogen, and phosphorus, while potassium levels remained unaffected.

Treated plots showed a marked shift toward neutral pH compared to the control (6.15), with the highest values in the T1 (50kg N<sub>14</sub>P<sub>23</sub> + Bio treatment) (7.41) and T2 (25kg N<sub>14</sub>P<sub>23</sub> + Bio treatment) (7.44) treatments conditions favorable for nutrient solubility and microbial activity.

The increase in soil pH, following application of the treatments is in agreement with Karapouloutidou and Gasparatos, and is due to the alkaline nature of the bio treatments used [43]. Soil moisture content also increased favorably, peaking in T1 (65%), followed by T2 (63%) and T3 (62%), and the least being control (58%) (Table 7). These changes suggest improved soil structure and water retention capacity, likely driven by organic matter dynamics enhanced by the treatments.

Nitrate nitrogen concentrations rose substantially, with T1 reaching 21 mg/kg almost double the control (T0) (11 mg/kg), while T2 (18 mg/kg) and T3 (13 mg/kg) bio treatments also showed significant improvements, indicating enhanced nitrogen cycling. Similarly, phosphorus availability was highest in the T1 plot (22.4 mg/kg), exceeding the control (18.3 mg/kg), which suggests enhanced phosphorus mobilization in response to the bio treatment (Table 7). These findings are consistent with those of Pacci et al., who reported increased soil phosphorus levels following the application of biostimulants [44]. Potassium levels showed no significant differences between biotreatments, though a slight increase in T3 plot (337 mg/kg) may reflect sufficient baseline K availability in chernozem soils, limiting further treatment-induced mobilization [45].

## Biotreatment Effect on Soil Microbial Biomass

Soil microbial activity, assessed through Fluorescein Diacetate (FDA) hydrolysis, increased over time in response to bio treatments, although the extent of stimulation varied by formulation. This highlights the potential for increased soil health given their longer-term use, a trend reported by Yakhin et al. [46]. The T2 (25kg N<sub>14</sub>P<sub>23</sub> + Bio treatment) consistently induced the highest FDA activity, reaching 250  $\mu\text{g g}^{-1} \text{h}^{-1}$  by day 90. Both T3 (Bio treatment) and T1 (50kg N<sub>14</sub>P<sub>23</sub> + Bio treatments) also enhanced microbial activity relative to the control but followed distinct temporal patterns. The control maintained the lowest FDA values (197  $\mu\text{g g}^{-1} \text{h}^{-1}$ ), indicating limited microbial stimulation in untreated soils.

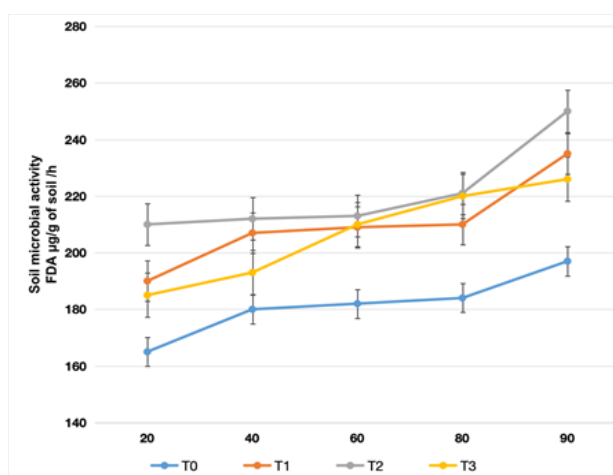


Fig. 4: Bio treatment impact on soil microbial activity (Measured by FDA Hydrolysis) Over Time

These findings underscore the potential of biostimulants and biologically derived fertilizers to enhance soil enzymatic function. While Soltaniband reported limited effects of biostimulants on FDA hydrolysis in strawberries, other studies show contrasting results [47]. Buragohain et al. observed increased FDA activity in rice soils, Khan et al. reported enhanced microbial growth from seaweed extracts, and Makova et al. reported improved FDA activity with a commercial stimulant alone and in combination with humic substances [48-50]. In addition to directly stimulating enzymatic activity, biostimulants may indirectly promote microbial proliferation by enhancing root growth [51], as observed in the present study. The resulting

increase in root biomass contributes to improved soil structure, aeration, and moisture retention conditions that are highly conducive to microbial activity [52-54].

The results of this study clearly demonstrate the potential of biostimulants and bio-derived fertilizers in addition to low dose mineral fertilizer to enhance the growth, yield, and soil health of spring wheat under the climatic conditions of Northern Kazakhstan. Compared to the conventional mineral fertilizer control, bio treatments combined with reduced mineral fertilization significantly improved agronomic performance and increased protein content in the harvested grains, without negatively affecting gluten or moisture levels, indicating quality preservation alongside yield enhancement.

## Conclusion

The successive two-year field results highlighted that T1 (50kg N<sub>14</sub>P<sub>23</sub>+ biotreatment) consistently produced the most superior effects across multiple parameters. In terms of physiological monitoring, NDVI values provided clear evidence of improved chlorophyll content and vegetative health particularly under T1 (50kg N<sub>14</sub>P<sub>23</sub> + Biotreatment). These observations align with enhanced nutrient availability and uptake efficiency facilitated by the applied formulations. Soil chemical analyses further confirmed the beneficial effects of the treatments. All biotreatments improved key indicators of soil fertility, including nitrate nitrogen, available phosphorus, soil pH, and moisture content.

In retrospect, the findings highlight the agronomic and ecological value of integrating low-dose mineral fertilization, bio stimulant and bio-derived fertilizer strategies into wheat cultivation systems. Such practices not only support crop productivity but also contribute to improved soil quality and resource-use efficiency. While we suggest that integrating bio-based inputs with reduced mineral fertilization, specifically the T1 treatment (50kg N<sub>14</sub>P<sub>23</sub> + biotreatment), presents a promising strategy for improving crop productivity and soil health in semi-arid wheat systems, our findings are limited to two growing seasons and a single location. Future research is suggested to focus on extended multi-seasonal trials and observation period in addition to economic analyses to fully assess their scalability and long-term impact across diverse agroecological zones.

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## Author's Contributions

Gani Stybaev and Aliya Baitelenova: Conceptualization, Funding acquisition, Methodology, Supervision, Data curation, Formal analysis, writing original draft, writing review and editing.

Kelvin Harrison, Rasushan Ramazanova, Meisam Zargar, and Adilbek Nogaev: Data curation, Formal analysis, Validation, writing original draft, writing review and editing.

Yeldos Kulzhabayev: Project administration, Resources.

## Ethics

This manuscript is original and contains unpublished material. All authors have read and approved the final version of the manuscript. The study did not involve human participants, animals, or any procedures requiring ethical approval. No ethical issues are associated with this research.

## Conflict of Interest

The authors declare no conflict of interest.

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